

Report for 2004AZ42B: Permeable Reactive Biobarriers for the Containment and Remediation of Acid Mine Drainage

- Articles in Refereed Scientific Journals:
 - Karri, S., Sierra-Alvarez R, Field JA. (2005). Toxicity of copper to methanogenic and sulfate reducing microorganisms. *Chemosphere*. (In press).
 - Karri, S., Sierra-Alvarez R, Field JA. (2005). Zero valent iron as an electron donor for methanogenesis and sulfate reduction in anaerobic sludge. (Under review).
 - Karri, S., Sierra-Alvarez R, Field JA. (2005). Treatment of acid mine drainage by sulfidogenic bioreactors. (In preparation).
- Dissertations:
 - Karri, S. (2004). Bioremediation of heavy metal using sulfate reducing bacteria. M.Sc. thesis. Department of Chemical and Environmental Engineering. The University of Arizona. Tucson, Arizona.

Report Follows

A. Problem and Research Objectives

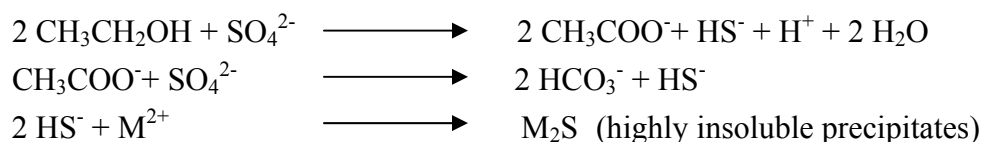
Statement of Critical Regional or State Water Problems

Due to a long history of mining and ore smelting in Arizona, abandoned mines and tailing piles threaten the State's surface and groundwater quality. Over 40 mines or metal-processing sites in Arizona are registered as CERCLIS (*Comprehensive Environmental Response, Compensation, and Liability Information System*) hazardous waste sites and 24 uranium mill processing sites are designated for remediation by the U.S. Department of Energy. The uncontrolled release of acid mine drainage (AMD) at many of these sites introduces acidity and elevated concentrations of sulfates, ferrous iron (Fe(II)), heavy metals and radionuclides into our water resources. In the 2002 report on the Status of Water Quality in Arizona (Arizona Department of Water Quality), metals/metalloids were the most important pollutant category responsible for impaired or non-attaining streams. Resource extraction (mining) was identified as the number one source for stream impairment. Metals and radiochemicals are also problematic groundwater pollutants responsible for 19 and 14%, respectively, of all index wells and target monitoring wells exceeding drinking water standards.

The overwhelming majority of Arizona's mining or tailings impacted sites are no longer in industrial operation. Consequently, cleanup funds are limited, leaving only low-cost extensive treatment or containment as viable options. Arizona State agencies, county- city- or tribal governments will be the most likely candidates for coordinating clean-up, restoration or containment operations.

Related Research

The most common methods for AMD remediation involve physical-chemical methods with high operating costs, and generation of bulky volumes of toxic sludges. Environmental biotechnologies offer interesting potentials for metal removal and recovery. Microbial processes for the removal of metals from aqueous streams generally rely on biosorption, reduction of metals to less soluble forms or chemical precipitation with biogenic products, e.g., phosphates or sulfides (8,9,14). This project considers the application of sulfate reducing bacteria (SRB) for the bioremediation of AMD. SRB are a diverse group of anaerobic prokaryotes characterized by their capacity to use sulfate (SO_4^{2-}) as a terminal electron acceptor. SRB are able to precipitate a wide spectrum of heavy metals found in AMD as sulfides minerals (3,6,7,5,23). SRB can also reduce the acidity- and sulfate levels in AMD (3,10). A simplified stoichiometric equation involving reduction of sulfate with organic substrates and precipitation of metals with formed sulfide can be represented as follows:



Where, M^{2+} is a divalent heavy metal cation

Removal of heavy metals by SRB has been applied for the removal of metals in AMD at pH values as low as 3 (13). Important examples include the mineralization of copper, zinc, cadmium, and arsenic as sulfides in SRB biofilms (23). The solubility product of most metal sulfides is extremely low enabling almost complete metal removal (eg., Cu, Zn, Cd) (12).

Bioreactor technology is well developed for the application SRB. High rate sulfidogenic bioreactors are already implemented at full-scale for the treatment of metals at a semiconductor plant (Phillips) and a zinc refinery (Budelco) in The Netherlands (1,22). However, for application in Arizona, the sulfate-reducing biotechnology should be adapted to applications in permeable reactive barriers, constructed wetlands or other extensive techniques that are low-cost and low-management.

PRBs provide an innovative, low-cost solution to prevent contaminant migration in groundwater. The technology is extremely simple involving trenches intercepting contaminated plumes. The trenches are filled with porous materials, nutrients and substrates to encourage the development of an active microbial population capable of metal removal (**Figure 1**). The reactive materials, which consist of organic substrates and/or zero valent iron, promote microbial-mediated sulfate reduction, the generation of hydrogen sulfide, and the subsequent precipitation of a wide spectrum of metal as well as metalloid contaminants as sulfide minerals. Several studies have previously considered reactive barriers that exploit the activity of sulfate-reducing microbial populations for the treatment of AMD or other heavy metal-laden leachates (2,7,15,19). In these studies, insoluble organic biomass was incorporated in the barrier for long-term release of electron donating substrate. When applied at field-scale to AMD from coal-mining, reduction in

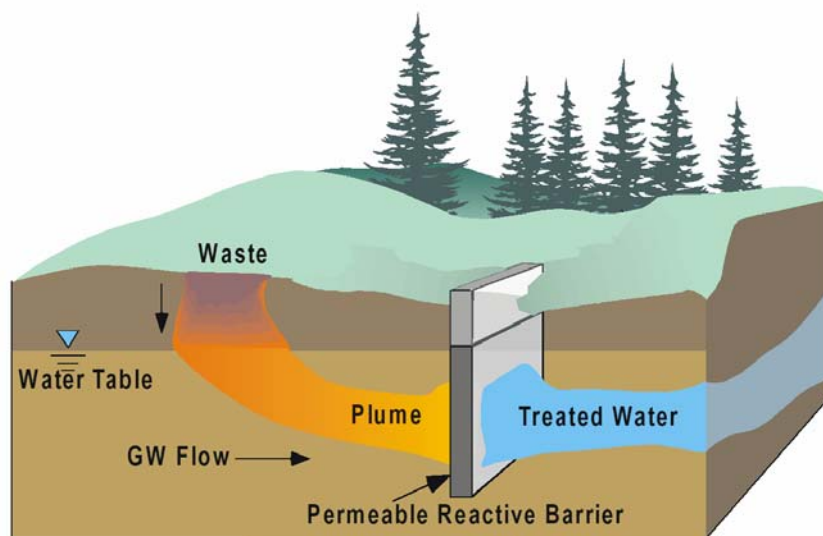


Figure 1. Schematic representation of a permeable reactive biobarrier.

(source: <http://207.86.51.66/download/rtdf/prb/reactbar.pdf>).

alkalinity and soluble iron could be demonstrated over several years (2).

Nature, Scope and Objectives:

The aim of this research is to develop low-cost extensive remedial strategies aimed at preventing water contamination from Arizona's mine or tailings impacted sites. Specifically, the project will examine the potential of permeable reactive biobarriers (PRBs) to prevent the spread of acidity, sulfates, and metals from acid mine drainage (AMD) to surface or groundwater. The study will also assess the applicability of zero valent iron as slow-release electron donor to promote sulfate-reducing microbial activity in PRBs. ZVI has been shown to be a suitable e-donor for methanogenesis and reductive dehalogenation (4, 16, 20), and thus is expected to function under sulfate reducing conditions. Sulfate reducing bacteria (SRB) have been observed in PRB based on zero valent iron materials (11). The release of ferrous iron (Fe(II)) will have the additional benefit of immobilizing sulfides, and thus physically removing sulfur from the plume.

The main tasks of the project are as follows:

Task 1: Assessment of Inhibitory Effects of Heavy Metals to Anaerobic Microorganisms.

Toxicity batch bioassays were conducted to evaluate the effect of heavy metals on the specific activities of SRB and methanogens. Hydrogen and acetate were utilized as the electron donors for the process and copper was the model compound. Cu is the most frequent heavy metal found in the AMD. Inhibitory concentrations were determined from the bioassays.

Task 2: Suitability of Zero Valent Iron for Sulfate Reduction and Methanogenesis.

Zero valent iron (ZVI) was examined in long-term batch experiments, carried out over a 3-month period, to test the hypothesis that ZVI can serve as an electron donor for SRB and for methanogenic microorganisms. Series varying the grade and particle size of ZVI were compared with negative controls lacking electron donor and positive controls supplemented with a soluble electron donor. Methanogenesis or sulfate elimination and sulfide formation were measured as a function of incubation time, depending on the assay. The most effective slow-release electron donor was selected for application in future research.

Task 3: Remediation of AMD in Permeable Reactive Bio-barriers.

Continuous flow studies were conducted in ethanol/acetate-fed bench-scale columns simulating the operation of sulfate reducing PRBs. The study evaluated the immobilization of three predominant metals found in AMD-impacted streams (Cu, Zn, Ni), starting with addition of copper and, later on, shifting to a cocktail of the three metals. The operation of the bioreactor supplied with the simulated AMD was compared with that of a control bioreactor (no metals in the influent) operated under the same conditions.

B. Methodology

Microorganisms

A sulfate reducing anaerobic granular sludge was obtained from a full-scale upward sludge blanket (UASB) reactor treating rayon fiber manufacturing wastewater (Twaron, Twente, The Netherlands). The sludge had an initial content of volatile suspended solids (VSS) of 7.24%. The maximum methanogenic activity of the Twaron sludge in assays utilizing acetate and hydrogen, as substrate was 26.9 and 85.2 mg CH₄-COD g⁻¹ VSS day⁻¹, respectively. The maximum sulfidogenic activity of the Twaron sludge in assays utilizing acetate and hydrogen, as substrate was 7.6 and 10.7 mg S-SO₄²⁻ reduced g⁻¹ VSS day⁻¹, respectively. The microbial cultures were elutriated to remove the fines and stored under nitrogen gas at 4°C.

Media for Bioassays

The anaerobic basal mineral medium (pH 7.2) used in methanogenic bioassays (*M-1*) contained (in mg L⁻¹): NH₄Cl (280); NaHCO₃ (5,000); K₂HPO₄ (250); CaCl₂•2 H₂O (10), MgCl₂•6 H₂O (183), yeast extract (100), and 1 mL L⁻¹ of trace element solution. The basal medium (pH 7.2) utilized in the sulfate reducing bioassays (*M-2*) consisted of (in mg L⁻¹): NH₄Cl (280); NaHCO₃ (5,000); K₂HPO₄ (600); NaH₂PO₄•2 H₂O (796), CaCl₂•2 H₂O (10), MgCl₂•6 H₂O (100), Na₂SO₄ (2960); the specific methanogenic inhibitor 2-bromoethane sulfonate (6,330), yeast extract (20), and 1 mL L⁻¹ of trace element solution. The trace element solution contained (in mg L⁻¹): H₃BO₃ (50), FeCl₂•4 H₂O (2000), ZnCl₂ (50), MnCl₂•4H₂O (50), (NH₄)₆Mo₇O₂₄•4H₂O (50), AlCl₃•6 H₂O (90), CoCl₂•6 H₂O (2,000), NiCl₂•6 H₂O (50), CuCl₂•2 H₂O (30), NaSeO₃•5 H₂O (100), EDTA (1,000), resazurin (200) and 36% HCl (1 mL L⁻¹).

Batch Bioassays

Different grades of ZVI were utilized in the bioassays as electron donors to test the slow release electron donating capacity. The various types of ZVI utilized were: < 10 micron (0.010 mm diameter), 325 mesh (0.044 mm particle diameter), 100 mesh (0.149 mm particle diameter) and an industrial sample of sieve size -8+50 mesh (average particle diameter of 1.129 mm). Initial experiments of sulfate reduction and methanogenesis were conducted with 46.6 g L⁻¹ of 325 mesh ZVI. Additional assays were later conducted to analyze the effect of particle diameter on the rate of electron releasing capability of ZVI for both sulfate reduction and methanogenesis. A ZVI concentration of 18.64 g L⁻¹ was utilized for these tests. Hydrogen was used as the electron donor in positive controls and was supplied as H₂/CO₂ gas (80/20, v/v) at 1.5 atm.

Methanogenic Test with ZVI. Shaken batch bioassays to test the effect of particle diameter on the rate of production of methane were conducted in 165 mL serum flasks. Anaerobic sludge (3 g VSS L⁻¹) was transferred to serum flasks with 28 mL basal medium M-1. ZVI was added at 18.6 g L⁻¹. The flasks were incubated overnight at 30±2°C to adapt the sludge to the medium conditions. On the following day, the flasks containing H₂ were reflashed with N₂/CO₂ (80/20,

v/v), and then pressurized with H_2/CO_2 (80/20, v/v, 1.5 atm), while all the other flasks were flushed with N_2/CO_2 for 3 min. All the flasks were incubated for 2 h. Methane, total iron and soluble iron were monitored periodically for the subsequent 75 days. The controls containing H_2 as an electron donor were reflushed after 355 and 736 h, respectively, for 3 min (80/20, v/v, 1.5 atm), after flushing first with N_2/CO_2 . At the same time periods, all the other flasks were reflushed with N_2/CO_2 to avoid build up of methane.

Sulfate Reduction Test with ZVI. Anaerobic sludge (1.5 g VSS L^{-1}) was transferred to 335-mL serum flasks containing 250 mL of medium M-2. In flasks containing H_2 as the electron-donor, 100 mL of basal medium was utilized instead. ZVI was added at 46.6 or 18.64 g L^{-1} , depending on the assay. The medium and the headspace were flushed with N_2/CO_2 gas (80:20, v/v) to exclude oxygen, and the bottles were sealed with butyl rubber septa. Flasks containing H_2 as electron donor were first flushed with N_2/CO_2 and then pressurized with H_2/CO_2 (80/20, v/v, 1.5 atm) for 3 min. The flasks were incubated overnight at $30 \pm 2^\circ\text{C}$ to adapt the sludge to the medium conditions. On the following day, the flasks containing H_2 , were reflushed with N_2/CO_2 and then pressurized with H_2/CO_2 (80/20, v/v, 1.5 atm) for 3 min. Sulfate and sulfide were monitored over the course of the experiment of 109 day duration. The controls containing H_2 were reflushed after 902 or 1744 h for 3 min (80/20, v/v, 1.5 atm), depending on the assay, after flushing first with N_2/CO_2 . Sample analysis for sulfate, sulfide, total and soluble iron were measured periodically.

Various controls (uninoculated controls, no-substrate controls, positive controls with H_2 as electron-donor) were included, for all the experiments. All flasks were sealed with butyl rubber stoppers and aluminum crimp seals, and they were incubated in a climate-controlled chamber at $30 \pm 2^\circ\text{C}$ in an orbital shaker (75 rpm). All assays were conducted in triplicate.

Bioreactors

Biological removal of heavy metals was investigated in two different sulfidogenic anaerobic bioreactors (each of volume 409 mL) continuously fed with a synthetic acid mine drainage, one reactor being the Control Reactor (CR) and the other being the Metal Reactor (MR). Both reactors were operated under similar influent conditions for a period of 73 days. Subsequently, the influent of MR was supplied with increasing concentrations of heavy metals. The reactors were placed in a climate controlled room at $30 \pm 2^\circ\text{C}$. The reactors were inoculated with 10 g VSS L^{-1} of the anaerobic sludge. **Figure 2** presents a schematic drawing of the

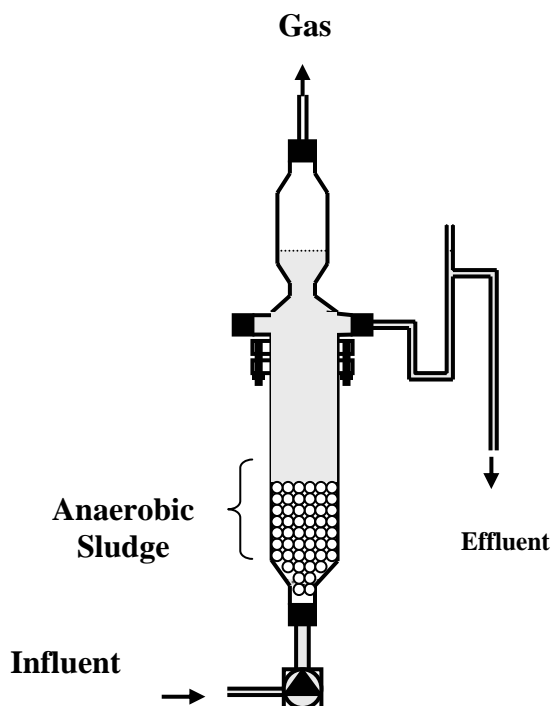


Figure 2. Schematic representation of the 0.5-L laboratory scale up-flow sludge bed reactors used in this study.

CR and MR systems. The reactors were maintained at an average hydraulic retention time (HRT) of 24 h.

The reactor medium was prepared using basal mineral medium M-1, Na_2SO_4 ($2,660 \text{ mg L}^{-1}$) and ethanol (490 mg L^{-1}). After a period of 134 days, acetate also added at concentrations ranging 180 to 250 mg L^{-1} . The chemical oxygen demand (COD) factors for ethanol and acetate are 2.089 and 1.067 , respectively. The pH value of the influent was decreased stepwise from 8.0 to only 4.5 . Both reactors were operated with the metal-free influent for 73 days, at which point copper (II) (as CuCl_2) was added to MR at a concentration of 10 mg L^{-1} . The concentration of copper was increased periodically to 20 mg L^{-1} (on Day 173); 50 mg L^{-1} (on Day 255); 100 mg L^{-1} (on Day 291). To simulate AMD conditions, the MR was supplied with a cocktail of heavy metals that contained: copper (100 mg L^{-1}), nickel (15 mg L^{-1}) and zinc (15 mg L^{-1}) (added as the respective chloride salts) from Day 343 to Day 393. The NaHCO_3 concentration in the influent medium during this period was increased to 1 g L^{-1} for providing an effective buffering of the system. The various periods of operations for the sulfidogenic reactors are presented in [Table 1](#).

Both reactors were monitored daily for influent and effluent pH, liquid volumetric flow rate, and gaseous methane flow rate. Reactor influent and effluent were sampled daily or every other day and analyzed for sulfate, ethanol, acetate and metal concentration (*i.e.*, Cu or Cu, Ni and Zn, depending on the experimental period). Effluent samples were also analyzed for sulfide concentration.

The methane production in the reactors was measured by liquid displacement using inverted 1-L serum flasks filled with 1 M NaOH solution to scrub H_2S and CO_2 from the biogas. The H_2S concentration in the biogas stream was calculated from the H_2S concentration in the liquid assuming equilibrium between the gas and liquid phases. CO_2 concentrations in the biogas were assumed to be 30% of the total methane flow rate.

For analyzing the changes in the microbial communities established in the bioreactors, samples for cloning and fluorescence *in situ* hybridization (FISH) analysis were also taken every time the influent metal concentration was changed. The results from these analyses are part of separate study and hence will not be reported here.

Analytical Methods

The acetate concentration in liquid samples from both the reactors, as well as the methane content in the headspace of the activity assay serum flasks was determined by gas chromatography using an HP5290 Series II system (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (GC-FID). The GC was fitted with a Nukol fused silica capillary column ($30 \text{ m length} \times 0.53 \text{ mm ID}$, Supelco, St. Louis, MO). The temperature of the column, the injector port and the detector was 140 , 180 and 275°C , respectively. The carrier gas was helium at a flow rate of 9.3 mL min^{-1} and a split flow of 32.4 mL min^{-1} . Formic acid ($22.5 \text{ } \mu\text{L}$ per mL of sample) was added prior to volatile fatty acid (VFA) analysis. Samples for measuring methane content ($100 \text{ } \mu\text{L}$) in the headspace were collected using a pressure-lock

gas syringe. Ethanol was analyzed by GC-FID using a DB-FFAP column (J&W Scientific, Palo Alto, CA). The temperature of the column, the injector port and the detector was 70, 180 and 275°C, respectively. The carrier gas was helium at a flow rate of 9.3 mL min⁻¹ and a split flow of 32.4 mL min⁻¹.

Sulfide was analyzed colorimetrically by the methylene blue method (17). Sulfate was determined by ion chromatography with suppressed conductivity using a DIONEX system equipped with a Dionex AS11-HC4 column (Dionex, Sunnydale, CA) and a conductivity detector. The mobile phase was 15 mM KOH at a flow rate of 1.2 mL min⁻¹. The column temperature was maintained at room temperature. The injection volume was 25 µL. Total Cu and soluble Cu in liquid samples were quantified with atomic absorption spectrometry. The total copper content in sludge sample of the metal reactor was measured following extraction of the samples with 10 mL of HCL (6.75 N) in a microwave digestion system (MDS2100, CEM Corporation, Matthews, NC) for 35 min. For analyzing soluble copper, all liquid samples were membrane filtered (0.40 µm) immediately after sampling. The samples were acidified with 2-3 drops of (5%, v/v) nitric acid to pH < 2 to prevent metal precipitation and adsorption to surfaces and stored in plastic vials for analysis. The copper, nickel and zinc concentration in liquid samples was analyzed by ICP-MS (Agilent 7500a system). The analytical system was operated at a Rf power of 1500 watts, a plasma gas flow of 15 L min⁻¹ and a carrier gas flow of 1.2 L min⁻¹. The pH was determined immediately after sampling with a Orion model 310 PerpHecT pH-meter with a PerpHecT ROSS glass combination electrode. Volatile suspended solids (VSS) were determined according to *Standard Methods for Examination of Water and Wastewater* (1998. Clesceri et al. (eds.), 20th Ed. Washington D.C., American Public Health Association).

Chemicals

Iron powder, (-325 mesh; 97% purity) and iron powder, (<10 µm, 99.9+%) was obtained from Sigma Aldrich (St. Louis, MO); Iron powder (100 mesh; 99.9%) was obtained from Mallinckrodt (Hazelwood, MO) and the industrial iron sample (-8+50 mesh; 98%) from Conelly GPM Inc, (Chicago). Specialty gases N₂/CO₂ and H₂/CO₂ (80/20, v/v) were delivered from US Air weld (Phoenix, AZ). Cupric chloride dihydrate (Cu(II); 100.2%) was obtained from Mallinckrodt (Hazelwood, MO); nickel chloride (99.3%) from Alfa Aesar (Ward Hill, MA); and zinc chloride (ZnCl₂) and sodium sulfate (99%+) from Sigma-Aldrich (St. Louis, MO). Ethanol (100%) was purchased from Aaper alcohol (Shelbyville, KY).

C. Principal Findings and Significance

Task 1 - Toxicity of copper to acetoclastic and hydrogenotrophic activities of methanogens and sulfate reducers in anaerobic sludge

Heavy metals could negatively impact anaerobic microorganisms in anaerobic sulfate reducing bioreactors utilized for metal removal. The objective of this study was to evaluate the inhibitory effect of copper to acetoclastic and hydrogenotrophic activities of methanogens and sulfate reducers in sludge obtained from a full-scale sulfate reducing bioreactor. The 50% inhibiting concentration (50%IC) of Cu(II) to acetoclastic and hydrogenotrophic methanogens was 20.7 and 8.9 mg L⁻¹, respectively (Figure 3). The 50%IC of Cu(II) to acetoclastic sulfate reduction was 32.3 mg L⁻¹. The hydrogenotrophic sulfate reducers were only inhibited by 27% at the highest concentration of Cu(II) tested, 200 mg L⁻¹, indicating a high level of tolerance. The soluble Cu(II) was observed to decrease rapidly in both the methanogenic and sulfate reducing assays. The highest level of decrease was observed in the hydrogenotrophic sulfate-reducing assay which was over 99% in 5 h. Thus, the production of sulfides during the sulfate reducing assays may have accounted in part for the higher tolerance of sulfate reducers to Cu²⁺ toxicity compared to methanogens in this study. The results of this study indicate that sulfate reducing biotechnologies would be robust at relatively high inlet concentrations of Cu(II).

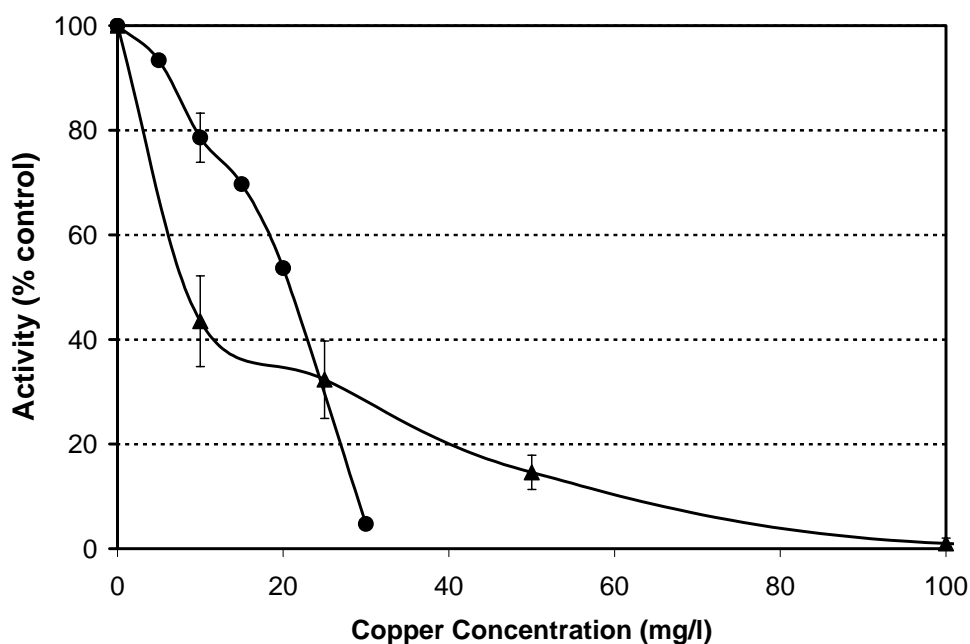


Figure 3. The role of the initial Cu(II) concentration on the methanogenic activity normalized with respect to the control in assays with either acetate (●) or hydrogen (▲) as the assay substrate.

Task 2 - Zero valent iron as an electron-donor for methanogenesis and sulfate reduction in anaerobic sludge

Zero valent iron (ZVI) is a reactive media commonly utilized in permeable reactive barriers. Sulfate reducing bacteria are being considered for the immobilization of heavy metals in PRBs. The purpose of this study was to evaluate the potential of ZVI as an electron donor for sulfate reduction in natural mixed anaerobic cultures. The ability of methanogens to utilize ZVI as an electron donor was also explored since these microorganisms often compete with sulfate reducers for common substrates.

Figure 4 illustrates the time course of the sulfate concentration in an uninoculated control; a control containing inoculum but no ZVI, and the complete treatment containing ZVI (325 mesh) and inoculum. Some sulfate was eliminated slowly from the two controls; however, the loss in sulfate concentration was distinctly greater and more rapid in the complete treatment. The results clearly indicate that ZVI was utilized by sulfate reducing bacteria.

Four grades of ZVI of different particle sizes (1.120, 0.149, 0.044 and 0.010 mm diameter) were compared as electron donor in batch bioassays inoculated with anaerobic bioreactor sludge.

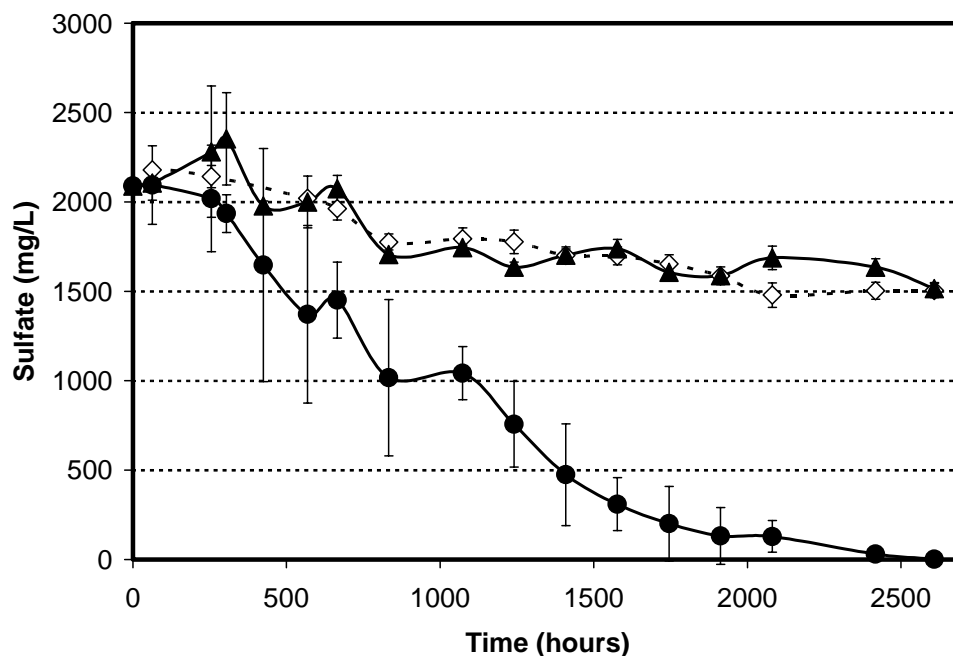


Figure @@. The time course of the sulfate concentration with 46.6 g L⁻¹ of ZVI (325 mesh) and 1.5 g VSS L⁻¹ of anaerobic sludge. Legend: (●), complete treatment with sludge and ZVI; (▲), endogenous sludge control; and (◇), uninoculated ZVI.

Methanogenesis was evaluated in mineral media lacking sulfate. Sulfate reduction was evaluated in mineral media containing sulfate and the specific methanogenic inhibitor, 2-bromoethane sulfonate. ZVI contributed to significant increases in methane production and sulfate reduction compared to endogenous substrate controls. The rates of methane formation or sulfate reduction were positively correlated with the surface area of ZVI. The highest rates of 0.310 mmol CH₄ formed (mol Fe⁰)⁻¹ d⁻¹ and 0.804 mmol SO₄²⁻ reduced (mol Fe⁰)⁻¹ d⁻¹ were obtained with the finest grade of ZVI (0.01 mm). The results demonstrate that ZVI is readily utilized as a slow-release electron donor for methanogenesis and sulfate reduction in anaerobic sludge; and therefore, has a promising potential in bioremediation applications.

Task 3 - Treatment of acid mine drainage by sulfidogenic bioreactors

High concentrations of heavy metals, sulfate and acidity are a frequent problem associated with AMD. Biogenic production of sulfide from sulfate reducing biosystems can be successfully utilized to remediate these acidic streams. Continuous-flow anaerobic reactors were designed to demonstrate the removal of heavy metals from a synthetic AMD with defined concentrations of sulfate and metals. A mixture of ethanol and acetate was used as the electron donating substrate for the system. Copper (II) concentrations from 10 to 100 mg L⁻¹ were tested. After successful removal of copper, biological removal of simulate AMD containing a heavy metal cocktail consisting of copper (100 mg L⁻¹), zinc (15 mg L⁻¹) and nickel (15 mg L⁻¹) was evaluated. Sulfate reducers were able to precipitate these heavy metals with efficiencies greater than 99.5% (Figures 5 and 6, Tables 2 and 3). The overall acidity of the system was reduced effectively from pH values as low as 4.5 in the influent to near neutral pH values in the treated effluent (Figure 7). During the final operation periods, about 1.42 and 1.03 g SO₄²⁻ L⁻¹ reactor day⁻¹ was removed in the control and metal bioreactors. Anaerobic sulfate reducing bioreactor systems have great potential to remediate high influent pH, sulfate and metal concentrations with high efficiencies.

Table 1. Periods of reactor operation^a .

Period	Days of Operation	Operational Conditions
Reactor 1 (Control Reactor)		
I	Day 0 - Day 73	Influent pH: 8.0; Steady state, sulfidogenic conditions (ethanol)
II	Day 74 - Day 133	Influent pH: 6.5
III	Day 134 - Day 221	Influent pH: 5.0; Addition of acetate: 197 mg COD L ⁻¹
IV	Day 222 - Day 393	Influent pH: 4.5; Addition of acetate: 267 mg COD L ⁻¹
Reactor 2 (Metal Reactor)		
I	Day 0 - Day 73	Influent pH: 8.0; Steady state, sulfidogenic conditions (ethanol)
II	Day 74 - Day 133	Influent pH: 6.5; Addition of 10 mg L ⁻¹ Cu (II)
III	Day 134 - Day 173	Influent pH: 5.0; Addition of acetate: 197 mg COD L ⁻¹ and 10 mg L ⁻¹ Cu (II)
IV	Day 174 - Day 221	Influent pH: 5.0; Addition of acetate: 197 mg COD L ⁻¹ and 20 mg L ⁻¹ Cu (II)
V	Day 222 - Day 255	Influent pH: 4.5; Addition of acetate: 267 mg COD L ⁻¹ and 20 mg L ⁻¹ Cu (II)
VI	Day 256 - Day 291	Influent pH: 4.5; Addition of acetate: 267 mg COD L ⁻¹ and 50 mg L ⁻¹ Cu (II)
VII	Day 292 - Day 342	Influent pH: 4.5; Addition of acetate: 267 mg COD L ⁻¹ and 100 mg L ⁻¹ Cu (II)
VIII	Day 343 - Day 393	Influent pH: 4.5; Addition of acetate: 267 mg COD L ⁻¹ and addition of 100 mg L ⁻¹ Cu (II); 15 mg L ⁻¹ of Ni (II) and Zn (II)

^a Ethanol Concentration: 0.9 ± 0.2 g COD L⁻¹; Sulfate Concentration: 0.6 ± 0.1 g S-SO₄ L⁻¹

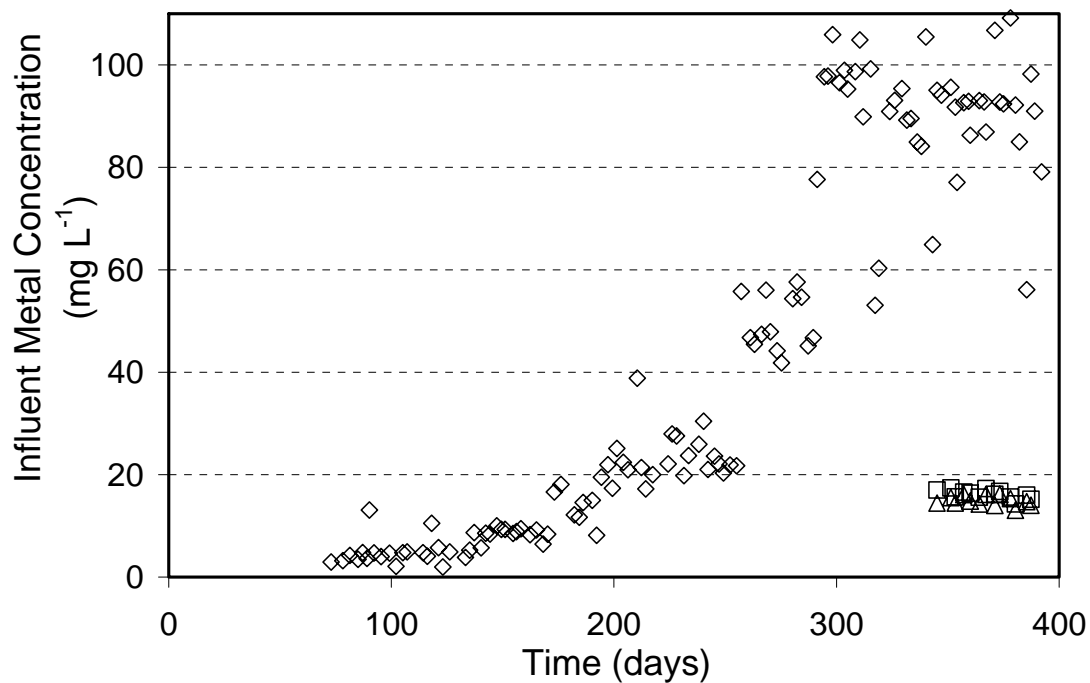


Figure 5. Concentration of heavy metals in the bioreactor influent: (◇) Cu, (□) Zn; (Δ) Ni.

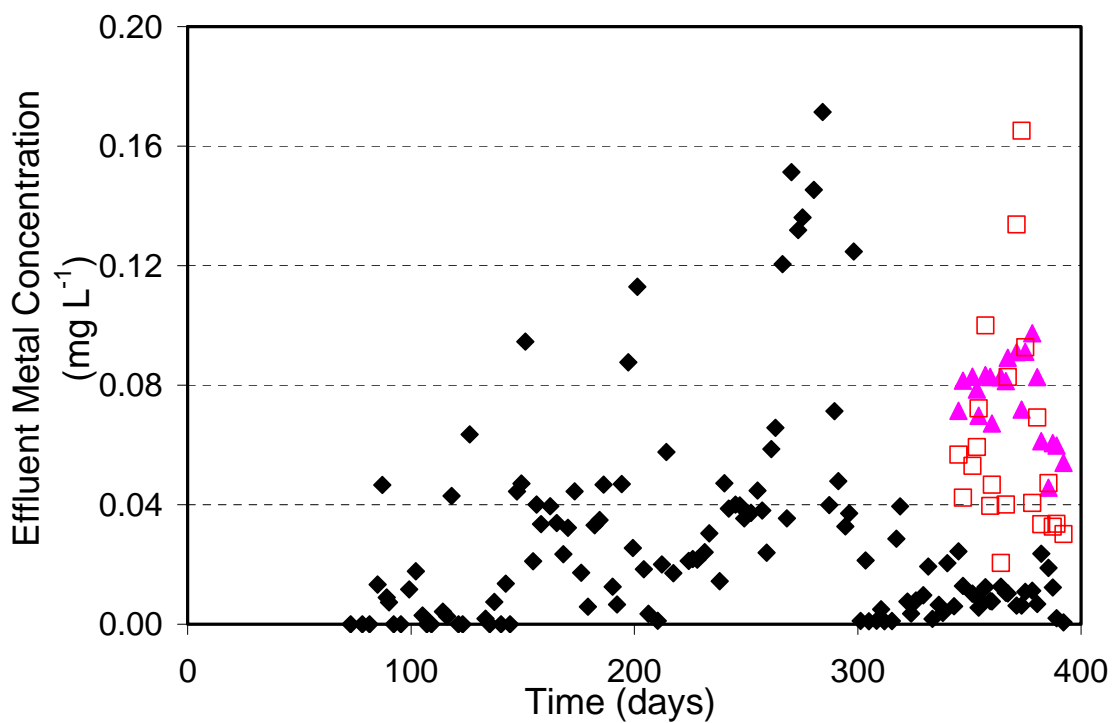


Figure 6. Concentration of heavy metals in the bioreactor effluent. (◆) Cu, (□) Zn; (▲) Ni.

Table 2. Average performance of the metal reactor (MR) during the various operational periods - Nickel and zinc data

Parameter	
Influent Zinc (mg L ⁻¹)	15.8 ± 0.8
Zinc Loading Rate (mg Zn L ⁻¹ d ⁻¹)	15.5 ± 1.9
Soluble Zinc Removal Efficiency (%)	99.2 ± 0.5
Total Zinc Removal Efficiency (%)	99.6 ± 0.2
Influent Nickel (mg L ⁻¹)	14.6 ± 0.6
Nickel Loading Rate (mg Ni L ⁻¹ d ⁻¹)	14.3 ± 1.8
Soluble Nickel Removal Efficiency (%)	99.3 ± 0.2
Total Nickel Removal Efficiency (%)	99.5 ± 0.1

Table 3. Average performance of the metal reactor (MR) during the various operational periods - Copper data.

Parameter	Period						
	II	III	IV	V	VI	VII	VIII
Influent Copper (mg L ⁻¹)	4.3±2.2	9.1±2.9	18.1±5.8	20.9±2.4	47.9±10.9	85.9±12.5	92.5±6.4
Copper Loading Rate (mg Cu L ⁻¹ d ⁻¹)	4.9±2.7	8.5±2.7	19.0±6.9	23.7±3.3	51.5±9.1	91.2±14.6	90.5±10.8
Soluble Copper Removal Efficiency (%)	N/A	N/A	N/A	N/A	N/A	N/A	100.0±0.0
Total Copper Removal Efficiency (%)	99.7±0.4	99.7±0.3	99.8±0.1	99.9±0.0	99.8±0.1	100.0±0.0	100.0±0.0

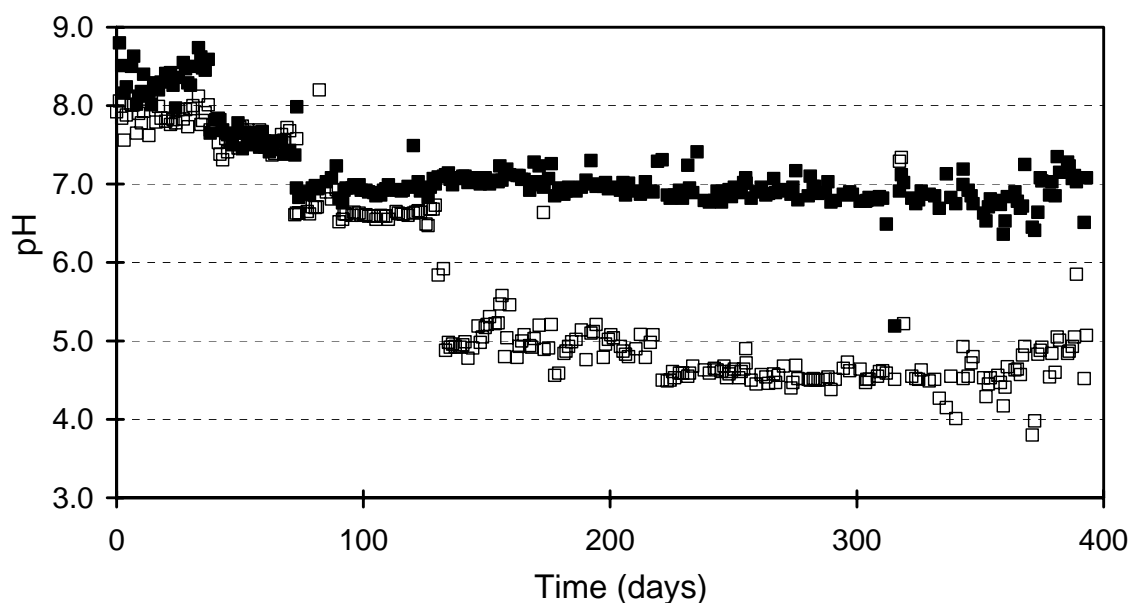


Figure 7. Influent (□) and effluent (■) pH values in the Metal Reactor as a function of time.

The results of this study indicate that there is a great potential for anaerobic sulfate-reducing bioreactor systems to remediate high influent pH, sulfate and metal concentrations with high efficiencies. Sulfate reducing biotechnologies proved robust at relatively high inlet concentrations of heavy metals. Furthermore, the results obtained confirm that sulfate reducing bacteria can utilize zero valent iron as an electron donating substrate. Methanogenic microorganisms, which often coexist and compete with SRB for available substrates were also able to use ZVI as electron donor. ZVI is an interesting slow-release electron donor to support sulfate-reducing activity in permeable reactive biobarrier systems

Acknowledgements

This research was supported in part by a grant from the USGS 104B Grant Program and by a National Science Foundation grant (R. S.-A., NSF-0137368 award). Undergraduate support was partly funded by grants from the University of Arizona Technology and Research Initiative Fund (S. Freeman, Undergraduate Water Fellowship Award); University of Arizona/NASA Undergraduate Internship Program and by a NSF- Research Experiences for Undergraduates grant.

References

1. **Barnes, L. J., F. J. Janssen, P. J. H. Scheeren, J. H. Versteegh, and R. O. Koch.** 1992. Simultaneous microbial removal of sulfate and heavy-metals from wastewater. Transactions of the Institution of Mining and Metallurgy Section C-Mineral Processing and Extractive Metallurgy **101**:C183-C189.
2. **Benner, S. G., D. W. Blowes, W. D. Gould, R. B. Herbert, and C. J. Ptacek.** 1999. Geochemistry of a permeable reactive barrier for metals and acid mine drainage. Environmental Science & Technology **33**:2793-2799.
3. **Benner, S. G., D. W. Blowes, and C. J. Ptacek.** 1997. A full-scale porous reactive wall for prevention of acid mine drainage. Ground Water Monitoring & Remediation **17**:99-107.
4. **Daniels, L., N. Belay, B. S. Rajagopal, and P. J. Weimer.** 1987. Bacterial methanogenesis and growth from CO₂ with elemental iron as the sole source of electrons. Science **237**:509-511.
5. **Diels, N., N. van der Lelie, and L. Bastieans.** 2002. New developments in treatment of heavy metal contaminated soils. Re/Views in Env. Science & Bio/Technology **1**:75-82.
6. **Eger, P.** 1994. Wetland treatment for trace-metal removal from mine drainage - the importance of aerobic and anaerobic processes. Water Science & Technol. **29**:249-256.
7. **EPA.** May 2002. Permeable reactive barriers. Interim summary report: Permeable reactive barriers using continuous walls to treat metals. U.S. Environmental Protection Agency. Office of Solid Waste and Emergency Response. Technology Innovation Office.
8. **Gadd, G. M.** 2000. Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Current Opinion in Biotechnology **11**:271-279.
9. **Gadd, G. M.** 2001. Microbial metal transformations. Journal of Microbiology **39**:83-88.
10. **Greben, H. A., J. P. Maree, Y. Singmin, and S. Mnqanqeni.** 2000. Biological sulphate removal from acid mine effluent using ethanol as carbon and energy source. Water Science & Technol. **42**:339-344.
11. **Gu, B. H., D. B. Watson, L. Y. Wu, D. H. Phillips, D. C. White, and J. Z. Zhou.** 2002. Microbiological characteristics in a zero-valent iron reactive barrier. Environmental Monitoring and Assessment **77**:293-309.
12. **Kim, S. D., J. J. Kilbane, and D. K. Cha.** 1999. Prevention of acid mine drainage by sulfate reducing bacteria: Organic substrate addition to mine waste piles. Environmental Engineering Science **16**:139-145.
13. **Kolmert, A., and D. B. Johnson.** 2001. Remediation of acidic waste waters using immobilised, acidophilic sulfate-reducing bacteria. J. Chemical Technology and Biotechnology **76**:836-843.
14. **Lovley, D.** 2000. Environmental microbe-metal interactions. ASM Press, Washington, DC.
15. **Ludwig, R. D., R. G. McGregor, D. W. Blowes, S. G. Benner, and K. Mountjoy.** 2002. A permeable reactive barrier for treatment of heavy metals. Ground Water **40**:59-66.

16. **Novak, P. J., L. Daniels, and G. F. Parkin.** 1998. Enhanced dechlorination of carbon tetrachloride and chloroform in the presence of elemental iron and *Methanosarcina barkeri*, *Methanosarcina thermophila*, or *Methanosaeta concillii*. *Environmental Science & Technology* **32**:1438-1443.
17. **Trüper, H. G., Schlegel, H. G.** 1964. Sulphur metabolism in thiorhodaceae: Quantitative measurements on growing cells of *Chromatium okenii*. *Antonie van Leeuwenhoek* **30**:225-238.
18. **Varga, G. A., and E. S. Kolver.** 1997. Microbial and animal limitations to fiber digestion and utilization. *Journal of Nutrition* **127**:S819-S823.
19. **Waybrant, K. R., C. J. Ptacek, and D. W. Blowes.** 2002. Treatment of mine drainage using permeable reactive barriers: Column experiments. *Environmental Science & Technology* **36**:1349-1356.
20. **Weathers, L. J., G. F. Parkin, and P. J. Alvarez.** 1997. Utilization of cathodic hydrogen as electron donor for chloroform cometabolism by a mixed, methanogenic culture. *Environmental Science & Technology* **31**:880-885.
21. **Webb, S. M., G. G. Leppard, and J. F. Gaillard.** 2000. Zinc speciation in a contaminated aquatic environment: Characterization of environmental particles by analytical electron microscopy. *Environmental Science & Technology* **34**:1926-1933.
22. **Weijma, J., C. F. M. Copini, C. J. N. Buisman, and C. E. Schultz.** 2002. Biological recovery of metals, sulfur and water in the mining and metallurgical industry. *In*: P. Lens, L. Hulshoff Pol, P. Wilderer, and A. T. (eds.), *Water Recycling and Resource Recovery in Industry: Analysis, Technologies and Implementation*. IWA Publishing.
23. **White, C., J. A. Sayer, and G. M. Gadd.** 1997. Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. *Fems Microbiology Reviews* **20**:503-516.